# Spatial distribution of flavor components and antioxidants in the flesh of 'Conference' pears and its relationship with postharvest pathogens susceptibility.

Laia Torregrosa[1,2,3], Gemma Echeverria[2], Josep Illa[1], Rosario Torres[2] and Jordi Giné-Bordonaba[2]

[1] Department of Computing and Industrial Engineering, University of Lleida, C. Jaume II, 69 25001, Lleida, Spain.

[2] Postharvest Programme, Institute for Food and Agricultural Research and Technology (IRTA), Parc Científic i Tecnològic Agroalimentari de Lleida, Edifici Fruitcentre, 25003, Lleida, Spain.

[3] Industrial Leridana del Frío, (ILERFRED, SL), C. Josep Segura Farré, 706, 25191, Lleida, Spain.

**Highlights**

- $C_2H_4$ and $CO_2$ production rates were similar along different sections of the flesh
- Sugars, malic and ascorbic acid contents in ‘Conference’ pear are spatial-dependent
- Higher amounts of fructose and malic acid may favor *R. stolonifer* growth
- Spatial susceptibility to fungal pathogens was related to the VOCs flesh content

## Abstract

The spatial distribution of dry matter, ethylene production, respiration rate, organic acids, sugars, antioxidants, volatiles and fungal (*Penicillium expansum* and *Rhizopus stolonifer*) growth was evaluated analyzing four different slices of 'Conference' pear flesh taken along an equatorial radius. A common spatial distribution trend was found for ethylene emission, $CO_2$ production, antioxidant capacity and total phenolic compounds with a minimum in the slice under the skin and a maximum in the slice near the core. Fructose, which was the dominant sugar followed by sucrose and glucose, showed a quasi-linear decreasing profile from the outer slice towards the core. Malic and ascorbic acid had the highest content in the outer slice while citric remained practically constant over the different slices. Twenty-nine volatile organic compounds (VOCs) were identified using solid-phase microextraction (SPME), yet only six of them showed significant differences between flesh slices. The content in VOCs was further related to the tissue susceptibility to the above-mentioned postharvest pathogens using a multivariate approach. Fruit flesh from inner sections was more prone to *P. expansum* whereas flesh from the slice under the skin presented the highest incidence of *R. stolonifer*. A Partial Least Square (PLS) model showed that *P. expansum* growth was negatively correlated with malic acid, dry matter content, 2-ethyl-hexanal and butyl hexanoate concentrations and *R. stolonifer* was negatively correlated to sucrose and some volatiles such as hexanal and 1-butanol. Based on the results from the PLS, selected volatiles naturally present in the pear flesh were tested *in vitro*, at different concentrations, in order to investigate their effectiveness to control blue mold caused by *P. expansum* and soft rot caused by *R. stolonifer*. A completely control of *P. expansum* was found with 2-ethyl-hexanal application and hexanal while 1-butanol showed a total fungicide effect against *R. stolonifer*. This study is a step towards a better understanding of how biochemical compounds are spatially distributed among different slices of 'Conference' pears as well as in the development of natural compounds to fight major postharvest pathogens in pear fruit.

**Keywords:** 2-ethyl-hexanal, fungicide**,** *Penicillium expansum*, phenolic compounds, *Rhizopus stolonifer*, VOCs

## 1 Introduction

Pear is one of the most important fruit produced in Europe, with 'Conference' cultivar as the most commonly grown in north east of Spain. 'Conference' is highly appreciated by consumers due to its flavor, juiciness and aroma (Saquet, 2018).

'Conference' pear as a climacteric fruit is a highly perishable product. The climacteric phase is characterized by a peak in ethylene production accompanied by a peak in fruit respiration. The burst displayed in the ethylene production is considered to set off biochemical and physicochemical processes (Moya-León et al., 2006; Rapparini and Predieri, 2003) leading to the biosynthesis of aroma compounds and stablishing the nutritional properties of the fruit.

The variability in aroma compounds of pear fruit is known to largely depend on the cultivar (Qin et al., 2012), maturity stage (Zerbini et al., 1993), agro-climatic conditions (Li et al., 2013) and storage conditions or postharvest handling (Zlatić et al., 2016). Volatile compounds, together with sugars and organic acids content (Defilippi et al., 2009), play an important role in fruit flavor. The major sugars in pears are fructose, glucose and sucrose (Colaric et al., 2006; Kolniak-Ostek, 2016; Lindo-García et al., 2019; Moriguchi et al., 2019) while malic and citric are the predominant organic acids in most pear cultivars. The ratio of sugar to organic acids is generally referred as a good indicator of flavor (Sha et al., 2011). However, scarce information is available on how volatile compounds, sugars and organic acids, are spatially distributed within the pear flesh. In other species such as peach, the volatiles concentration has been reported to notably differ from skin to flesh (Aubert and Milhet, 2007).

Despite present at relatively low concentration, pears are also a source of ascorbic acid (AsA) (Galvis Sánchez et al., 2003) and other bioactive compounds, including polyphenols, which positively contribute to human health. AsA content in 'Conference' pears changes during the fruit development and postharvest handling (Veltman et al., 2000) and higher concentration of this compound within the pear flesh has been linked to lower incidence of core browning in 'Conference' (Veltman et al., 1999) as well as superficial scald in 'Blanquilla' pears

(Larrigaudière et al., 2016). Phenolic compounds also contribute to the fruit aroma and flavor (Imeh and Khokhar, 2002) and thanks to their anti-inflammatory and antimicrobial activity, can help to prevent human diseases (Liaudanskas et al., 2017).

Pear major losses take place during the postharvest phase being mainly caused by physical, physiological and pathological induced-changes. The main postharvest diseases of pears are caused by *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* (Sardella et al., 2016). Traditionally, pears have been treated with chemical fungicide in order to control postharvest decay. In the last years, new alternatives to curtail fungal growth such as the application of natural compounds, including those emitted by pears, have also been studied. Neri et al. (2006b), applied 2-hexanal vapors to satisfactorily control blue mold growth caused by *P. expansum* and, Alla et al. (2008) applied cinnamaldehyde vapors to control soft rot caused by *R. stolonifer*. Indeed, the antifungic or fungistatic activity of a range of volatiles is well documented (Mari et al., 2016, 2002; Neri et al., 2006a; Sivakumar and Bautista-Baños, 2014). However, whether the concentration of these 'antifungic' compounds along the pear flesh can account to improve resistance to certain fungal postharvest pathogens is still elusive.

Accordingly, the aims of the present study were: 1) To investigate the spatial distribution of the main flavor components and antioxidants in the flesh of 'Conference' pears. 2) To determine the behavior of flesh samples from different spatial positions artificially inoculated with *P. expansum* and *R. stolonifer* 3) To evaluate the protective effect of some naturally occurring volatile compounds against both pathogens.

## 2 Materials and methods

### 2.1 Plant material and experimental design

'Conference' pears (*Pyrus communis L.*) were harvested in August 2018 from a commercial orchard near Lleida (NE of Spain). Fruit was picked up at optimum commercial maturity according to local growers recommendations which are basically assessed in terms of firmness and sugars content (firmness≈ 55-65 N and total soluble solids >13 %). No pre-harvest fungicide treatments were applied later than 30 days prior the commercial harvest. Thereafter, fruit were transported to IRTA facilities where 108 fruit free from defects and uniform size were selected and divided in 3 groups of 20 fruit each plus 2 groups of 24 fruit each. One group of 20 fruit was used to evaluate the dry matter content, sugars, organic acids, antioxidant capacity and phenols. Another group was used to evaluate ethylene production and respiration, and the last group of fruit was used to evaluate the VOCs content. The 2 groups of 24 fruit were used to evaluate the growth ability of *P. expansum* and *R. stolonifer* along different spatial locations.

From each fruit a pulp cylinder in the radial direction, equatorial zone, from the outside of the fruit to the heart was extracted (Fig. s1). Each cylinder was 11 mm in diameter and 24 mm in length. Then, the peel was removed, and the cylinder was cut into 4 equal slices, 6 mm high each, named I, II, III and IV and corresponding to the 4 spatial positions considered in this study (Supplementary Figure 1; Outer slice (slice 'I') until the core (slice 'IV')).

**2.2 Dry matter content**

The dry matter content profile was determined in 20 fruit, 4 replicates of 5 fruit each. Five slices per each location were placed in a petri dish, weighted ($m_{0i}$) and immediately frozen with liquid nitrogen. Slices were lyophilized for 72 h. After this time, each petri dish was weighted ($m_{1i}$) and the dry matter content was evaluated according to the formula: $(m_{1i}/m_{0i})\cdot 100$.

**2.3 Ethylene production and respiration**

Ethylene production and respiration were measured by enclosing 5 slices per each location in airtight tubes of a known volume (4 replicates) and placed in an acclimatized chamber at 20 ºC for two hours. After that time, ethylene concentration was measured by removing 1 mL of gas sample from the headspace of the tube and injecting it into a gas chromatograph fitted with a FID detector (Agilent Technologies 6890, Wilmington, Germany) and an alumina column 80/100 (2 m × 3 mm) (Teknokroma, Barcelona, Spain) as described by (Giné-Bordonaba et al., 2014). Oxygen and carbon dioxide concentrations within the tubes were measured with an $O_2/CO_2$ gas analyzer (CheckPoint $O_2$/$CO_2$, PBI Dansensor, Ringsted, Denmark). Gas $i$ ($i = O_2, CO_2$, ethylene) production rate, $r_i$ ($mol_i\ kg^{-1}\ h^{-1}$), was then calculated using Eq. (1),

$$r_i = \frac{\Delta P_i \cdot V_g}{R \cdot T \cdot M_f \cdot \Delta t}, \tag{1}$$

where $\Delta P_i = P_i^t - P_i^0$ (Pa) is the difference between the initial partial pressure, $P_i^0$ and the partial pressure $P_i^t$ after time $\Delta t$ (h), $V_g = V_0 - V_f$ ($m^3$) is the gas volume inside the closed tube obtained as the difference of the tube capacity $V_0$ and the volume occupied by the slices $V_f$, $R$=8.314 J $K^{-1}$ $mol^{-1}$ is the universal gas constant, $T$ (K) is the absolute ambient temperature and $M_f$ (kg) is the mass of slices inside the tube. Initial partial pressure of ethylene and $CO_2$ were assumed to be zero, while initial $O_2$ partial pressure was assumed to be $0.21 \cdot 10^5$ Pa. The respiratory quotient, RQ, was calculated as the molar ratio of $CO_2$ produced to $O_2$ consumed by the fruit, $RQ = -r_{CO2} / r_{O2}$.

## 2.4 Determination of fruit sugar content

Lyophilized slices used in dry matter content determination were ground with a stainless-steel blender and 100 mg of the powder were used for sugar content determination. Glucose, fructose and sucrose were extracted from lyophilized material as described by Giné-Bordonaba and Terry (2010). Briefly, 100 mg of lyophilized sample were dissolved in 2 mL of 62.5 % (v/v) aqueous methanol solvent and placed in a thermostatic bath at 55 °C for 15 min, mixing the solution with a vortex every 5 min to prevent layering. Then, samples were centrifuged at 20 000 *g* for 7 min at 20 °C. The supernatant from each extraction was recovered and used for enzyme-coupled spectrophotometric determination of glucose and fructose (hexokinase/phosphoglucose isomerase) and sucrose (β-fructosidase) as described by Famiani et al. (2012) using commercial kits (BioSystems S.A., Barcelona, Spain) and following the manufacturer instructions. All results are expressed on a fresh weight basis.

## 2.5 Determination of fruit organic acid content

Extracts for malic and citric acids determination, were prepared as described in Giné-Bordonaba and Terry (2010) with some modifications. One hundred mg of lyophilized frozen fruit tissue from each location were added to 2 mL of HPLC-grade water. Samples were kept at room temperature (20 °C) for 10 min and then centrifuged at 20 000 *g* for 7 min at 20 °C. The supernatant from each extraction was recovered and used for enzyme-coupled spectrophotometric determination of malic (L-malate dehydrogenase) and citric (citrate lyase / malate dehydrogenase) acids as described by Famiani et al., (2012) using commercial kits (BioSystems S.A., Barcelona, Spain) and following the manufacturer instructions.

Ascorbic acid (AsA) was determined using the freeze-dried material described above. One hundred mg of freeze-dried fruit slices were diluted in 2 mL of 3% (v/v) meta-phosphoric acid (MPA) and 8% (v/v) acetic acid aqueous solvent, mixing the solution for 1 min with a vortex. Then, the samples were centrifuged at 24 000 *g* for 22 min at 4 °C. The supernatants of each sample were filtered through a 0.45 μm filter for High Performance Liquid Chromatography (HPLC) (Millipore, Bedford, MA, USA) and used for HPLC-UV determination as described by Collazo et al. (2018). All results are expressed on a fresh weight basis.

**2.6 Determination of fruit antioxidant capacity and total phenolic content**

Fruit antioxidant capacity and total phenolic compounds (TPC) were quantified from the freeze-dried material used in the dry matter content determination, as described earlier (Giné-Bordonaba and Terry, 2008). One hundred mg of freeze-dried fruit sample were mixed with 2 mL of 79.5% (v/v) methanol and 0.5% (v/v) HCl aqueous solvent. Sample extraction was held at 20 °C, mixing the solution every 15 min with a vortex (Giné-Bordonaba and Terry, 2016). From the same extract, TPC was measured by means of the Folin-Ciocalteu method calculated from a Gallic Acid Equivalent (GAE) curve and total antioxidant capacity was measured by the Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Szeto (1999). All results are expressed on a fresh weight basis.

**2.7 Spatial distribution of volatiles in pears**

Headspace solid-phase microextraction (HS-SPME) was used to extract and to determine the concentrations of volatile compounds along the cylinder of pear flesh. SPME fibers coated with a 65-μm layer of polydimethylsiloxane–divinylbenzene (65 μm PDMS/DVB; Supelco Co., Bellefonte, PA, USA) were used. Fibers were activated before sampling according to the manufacturer's instructions.

Five slices per each spatial location and per replicate (4 replicates) were frozen in liquid nitrogen, crushed together and immediately transferred to −80 °C storage until the volatile compounds were analyzed. For each extraction, 5 g of homogenized sample per location were placed in a 20 mL screw-cap vial containing 2 g of NaCl to facilitate the release of volatile compounds. Prior to sealing the vial, 2 μL of 0.03 mL $L^{-1}$ 3-nonanone was added as an internal standard, and the solution was mixed with a glass rod. The mixture was incubated and agitated at 40 ºC during 20 min. Afterwards, the SPME fiber was injected into the headspace and exposed for 30 min at 40 ºC to absorb the volatiles as described by Qin et al. (20129. Volatile compounds were subsequently desorbed as described by Iglesias et al. (2018) and results expressed on a fresh weight basis.

**2.8 Fungal growth evaluation in pear tissue**

Both strains used in this study, *P. expansum* (CMP-1) and *R. stolonifer* (RSF) belong to the collection from the Postharvest Pathology group of IRTA (Lleida). They were the most aggressive isolates capable of infecting pome fruit, respectively. Conidial suspensions were prepared by rubbing the surface of 7 to 10-day-old cultures grown on potato dextrose agar (PDA) with sterile water containing 0.01 % (w/v) Tween-80 using a sterile glass rod. Concentration of each fungus was determined using a haemocytometer and prepared to obtain $3 \cdot 10^4$ conidia $mL^{-1}$ of *P. expansum* and $1 \cdot 10^3$ conidia $mL^{-1}$ of *R. stolonifer*.

Two groups of 24 fruit (8 replicates, 3 fruit each) were used to evaluate the growth of fungi. The first group was used to evaluate the severity and incidence of *P. expansum* and the second the incidence of *R. stolonifer.* Fruit were disinfected with 0.525% (v/v) sodium hypochlorite (NaClO) for 5 minutes and cleaned five times with tap water. Once dried, a pulp cylinder of the fruit was extracted and cut into 4 slices as explained in the plant material and experimental design section.

Each slice of the first group was inoculated with 5 µL of *P. expansum* and the ones of the second group were inoculated with 5 µL of *R. stolonifer*.

*P. expansum* incidence was evaluated by measuring the diameter of fungus growth and severity infection was evaluated as the % of mycelial presence on slices regarding the total of infected samples. *P. expansum* incidence was evaluated after 72 h post the inoculation while *R. stolonifer* incidence was measured after 44 h post the inoculation.

### 2.9 Evaluation of fungistatic or fungicide activity of synthetic pear volatiles *in vitro*

Fungistatic and fungicide activity of the four most representative VOCs found in the Principal Component Analysis (PCA) of detected pear volatiles was evaluated as reported by Gotor-Vila et al. (2017) with some modifications. Briefly, pure standards of these four volatiles were purchased from Sigma-Aldrich (Madrid, Spain) and individually tested for suppressing mycelial growth of target pathogens. For this purpose, 10 µL of conidial suspension containing each pathogen were placed in the center of petri dishes containing PDA. Then, a paper filter (85 mm diameter) containing an aliquot of pure compound was positioned inside the cover of the dishes and the petri dishes were immediately sealed with parafilm and incubated at 25 °C. The aliquots of pure compounds introduced in the petri dishes were: 5, 10, 20, 40, 80, 160 and 320 µL corresponding to 0.027, 0.055, 0.11, 0.22, 0.44, 0.88, 1.76 mL $L^{-1}$ headspace, respectively. Measures for *P. expansum* were made after three, four, five and seven days post the inoculation and *R. stolonifer* after one, two and three days. The sample unit was represented by four replicates for each dose and pathogen and dishes with paper filter with water at 1.76 mL $L^{-1}$ were used as control. The percentage of mycelial inhibition (PMI) of fungal growth was calculated after 5 and 3 d from inoculation for *P. expansum* and *R. stolonifer,* respectively. Percentage mycelial inhibition (PMI) was determined according to the formula (%)=$[(d_c-d_t)/d_c)]\cdot 100$, where $d_c$ is the diameter growth average of control and $d_t$ is the treatment diameter average (Li et al., 2016). The effect of VOC's on fungus were tested by determining the effective concentration values that reduced the mycelial growth by 50% ($EC_{50}$) as reported by Alexander et al. (1999).

## 2.10 Statistical analyses

Means were compared by analysis of variance (ANOVA). When the analysis was statistically significant, the Tukey's Honestly Significant Difference (HSD) test at $P \leq 0.05$ was performed for separation of means.

A hierarchical cluster analysis dendrogram was done applying Ward method of minimum variance. The objective function was the error of the sum of the squares or variance (Ward, 1963). The dendrogram and the constellation graph were constructed in order to establish a preliminary relationship between sugars, organic acids and antioxidants in order to find relationships between different pear 'Conference' slices spatially distributed. The analyzed data included the 4 slices along the spatial distribution (I, II, III and IV) and 40 variables representing the components being analyzed.

Two partial least square (PLS) regression models were used to correlate organic acids, sugars, antioxidants and volatile compounds (as X variables or explanatory variables) with fungal infections as response variables, *P. expansum* as ($Y_1$) and *R. stolonifer* as ($Y_2$). The non-linear iterative partial least squares (NIPALS) algorithm was used for computing the first few factors. KFold validation was used to select the number of factors that minimize the Root Mean PRESS statistic. As a pre-treatment, data were centered and weighed by the inverse of the standard deviation of each variable in order to avoid dependence on measured units. All analyses were carried out with the PLS platform of JMP® 13.1.0 SAS Institute Inc. (SAS, 2013).

# 3 Results and discussion

## 3.1 Dry matter content, ethylene emission and respiration

Dry matter (DM) of pip fruit is basically formed by carbohydrates (90 %) (Travers et al., 2014), in soluble and insoluble forms, and the remaining part are mainly organic acids (Suni et al., 2000). Our results showed that the DM content was minimum in slice II and III but with no significant differences between them (p=0.1891) (Fig. 1A). The average of DM content reported herein (17.8 %) was in accordance with the ones reported by Costa et al. (2015) in pear fruit from four different varieties (average 17.9 %). The moisture content profile, which is its complementary ($m_c$=100-DM), had thus a maximum in slice II, which can be explained by the fact that moisture diffuses outwards to the fruit surface at a higher flux rate than it does inwards, to the core of the fruit, hence resulting in a lower gradient towards the center.

Several studies have already analyzed the ethylene emission of whole pears at different maturities, temperatures and storage periods (Knee, 1987; Lindo-García et al., 2019; Villalobos-Acuña and Mitcham, 2008) as well as its respiration rate (Ho et al., 2018; Lammertyn et al., 2001; Saquet and Streif, 2017). To our knowledge no studies are available investigating the spatial distribution of ethylene production and respiration rates in pears. The ethylene production profile (Fig. 1B) presented a minimum at intermediate slices, II and III, with a significant increase towards the core. A similar profile, but with a better defined minimum at slice III, was found in the respiration rate (Fig. 1C). Our results showed a relatively poor correlation between respiration rate and ethylene production ($r^2$=0.546) likely due to the different diffusivity of both compounds (ethylene and $CO_2$) within the pear flesh. Rudell et al. (2000) found that ethylene production had a maximum in the carpellary tissue in ‘Fuji’ apple at all harvest dates, which is in accordance to our results found for the inner slice (referred as IV). Moreover, Rudell et al. (2000) reported a minimum in $CO_2$ production in the hypanthial tissue, hence also in accordance with our results (Fig. 1C).

### 3.2 Sugar and organic acid composition

Fructose, glucose and sucrose are known to be the main sugars in ‘Conference’ pear fruit and according to Colaric et al. (2007), in general, fructose represents more than 50 % of the pear sugar content. Our results are in accordance with this statement, fructose accounted for 60 % of the total sugar content, but clearly showed that these sugars were not uniformly distributed within the flesh of the fruit. Fructose content showed a quasi-linear decreasing profile with content values in the inner slice (slice IV Fig. 2A) about 40 % lower than in the outer slice, while sucrose showed an opposite trend with its lowest values under the fruit skin. Glucose content was minimum at slice

II (Fig. 2A) and significantly higher ($p > 0.022$) in the slice near the core (slice IV). Measured fructose values, 46.3 g $kg^{-1}$ as weighted average, were similar to the ones reported by Colaric et al. (2007) for 'Conference' pears harvested in 2004, however, these values were 1.5-fold lower than the ones obtained in the same study for fruit harvested in 2005. The measured glucose content (11.6 g $kg^{-1}$, weighted average) was nearly 2-fold higher than the values reported by Colaric et al. (2007) in fruit harvested in 2004 and Hudina and Štampar (2004) in Williams pears. Hudina and Štampar (2004) reported that the fruit sugar content was affected by climatic and soil conditions leading to differences as high as 50 %.

Malic acid is the predominant organic acid in 'Conference' pears followed by citric acid (Hudina and Stampar, 2000). The ratio between malic acid content and citric correlates with sensory perception of fruit taste (Colaric et al., 2007). In our measurements (Fig. 2B) malic was the predominant acid (3.6 g $kg^{-1}$ as weighted averages) and its distribution profile presented a minimum in slice II. Hudina and Štampar (2004) reported similar results (3.7 g $kg^{-1}$) for 'Conference' pears harvested at south-east of Slovenia. Kou et al., (2014) reported that malic acid content in the peel (3.6 g $kg^{-1}$) of 'Huang guan' pear was higher than in the pulp (2.2 g $kg^{-1}$) which is in line with our results. Citric spatial distribution followed a similar trend than the one observed in malic acid content although no significant differences were found between slices (Fig. 2B). Citric acid (1.2 g $kg^{-1}$ as weighted average) was 2.5-fold lower than malic acid in all slices.

In our study, only slice 'IV' had the lowest AsA content and showed significant differences if compared to the other slices ($p=0.0393$) (Fig. 2B). Johnson et al. (2013) found that AsA content in pulp (0.093 g $kg^{-1}$) of '*Citrullus Lanatus*' watermelon was higher than in rind and seed (0.076 and 0.053 g $kg^{-1}$, respectively). AsA content and fructose showed a quite good correlation with $r^2=0.764$. This result was in agreement with that found by Franck et al. (2003) who reported that AsA and fructose content had a similar pattern in 'Conference' pear, suggesting a close relationship between both components.

**3.3 Antioxidant capacity and total phenolic compounds**

According to different studies, pear fruit has beneficial health effects, protecting against different diseases, thanks to its antioxidant properties (Imeh and Khokhar, 2002; Kolniak-Ostek, 2016; Liaudanskas et al., 2017). Even though antioxidant capacity and total phenolic compounds in pears are low when compared to other fruit such as berries (Määttä-Riihinen et al., 2004), orange, kiwifruit and apples (Wang et al., 1996), the contribution of pear to the daily consumption of antioxidants and phenolics is relatively high (Chun et al., 2005). If compared to apples, total phenolic content in pear flesh is 3-fold lower (Leontowicz et al., 2002) and great variability exist among different pear cultivars (Brahem et al., 2017).

To our knowledge, little information is available about how antioxidant capacity and TPC are distributed along the flesh of fruit, and especially in pear. The fruit antioxidant capacity (1210.5 mg $Fe^{3+}$ $kg^{-1}$ as weighted average) had a minimum in slice III with a sharp increase in the slice near the core (Fig. 2C).

A similar profile was also found for TPC content (Fig. 2D). Imeh and Khokhar, (2002) analyzed TPC in different apple, pear and stone fruit cultivars and reported that 'Conference' pear had the lowest values (3023 mg $kg^{-1}$ GAE). However, their values were two-fold higher than that obtained in this study. This could be because in their analysis they included the peel, which is reported to have higher amounts of TPC.

### 3.4 Volatiles spatial distribution

While several studies have been focusing on 'Conference' pear volatiles emission under different circumstances (Goliáš et al., 2015; Hendges et al., 2018; Saquet, 2017) no information is available describing the VOC's concentration in different locations inside the pear flesh. Aubert and Milhet (2007) investigated the distribution of VOCs in different parts of a white-fleshed peach (cv. Maura) and found that volatiles content in skin were significantly higher than in flesh.

In our study twenty-nine volatile compounds were identified and quantified in the different locations of the slices in 'Conference' pear (Table s1). These volatile compounds included 16 esters, 6 alcohols, 3 aldehydes, 2 terpenoids, 1 acid and 1 ketone. Esters play an important role providing a characteristic fruity aroma (Zlatić et al., 2016) when volatiles are released from intact fruit. However, when fruit is cut or crushed different enzymatic processes can be activated, some of which are extremely rapid once cellular disruption begins (Rapparini and Predieri, 2003). In this context, aldehydes are major components in pulp extracts, but not in the headspace of intact pears.

Our research showed that hexanal was the main volatile detected with its highest concentration in the 'II' slice (140 µg $kg^{-1}$) but with no significant differences between locations of the slices (p=0.1278). Aldehydes are known to be the main responsible of grassy aroma (Zlatić et al., 2016) and green flavor (Rapparini and Predieri, 2003). Besides being a typical fruit volatile, hexanal is also formed when cellular structures are disrupted (Clark et al., 2014) and hence this compound is detected at its highest concentrations in fresh-cut fruit or when using similar methodologies to the one described herein (SPME);. For instance, Rizzolo et al. (2005), found that hexanal was one of the main volatile in 'Conference' pears under controlled atmosphere and it was the most prominent in odor units. Lindo-García et al. (2019) also found that hexanal was the principal aldehyde in 'Blanquilla' pears during on and off-tree ripening. Similarly, Makkumrai et al. (2014) reported that hexanal was the main aldehyde in 'Barlett' pears stored at 20 ºC for 11 d and Horvat et al. (1992) found that hexanal was one of the main volatiles in five Asian pear cultivars. All these studies used similar methodologies as the one described in this study.

The main ester detected was butyl butanoate which has been already reported as an impact volatile in 'Conference' pears (Rizzolo et al., 2005). Even though, no significant differences between locations of the slices were found, its maximum concentration was found in slice B. Butyl butanoate is largely known to contribute to sweet or fruity odors.

From the 29 identified volatiles only six presented significant differences between locations of the slices; butyl acetate, 2-ethy-hexanal, 3-methylbutyl 3-methyl-butanoate, (E)-2-hexenyl acetate, hexyl butanoate and hexyl 2-methylbutanoate. Some of these compounds have been previously identified as important character-impact volatiles in whole 'Conference' pears (El

Hadi et al., 2013; Saquet, 2017; Torregrosa et al., 2019) contributing, among others, to sweet and fruity odors. The spatial distribution of flavor components and antioxidants along the flesh of pear fruit may be of use to the fresh-cut industry to supply fruit with improved flavor and nutritional value by selecting not only the appropriate fruit but also specific parts of it.

### 3.5 Susceptibility to *P. expansum* and *R. stolonifer* along the pear flesh

*P. expansum* and *R. stolonifer* fungus are important destructive fungal pathogens of pome fruit. Many studies analyzed blue mould and soft rot in entire pears (López et al., 2015; Neri et al., 2010). However, no information is available about the fungal growth on flesh from different locations in 'Conference' pear.

*P. expansum* showed an incidence of 100 % in all locations of the evaluated slices, in contrast severity was significantly different between slices ($p < 0.001$), slice (I) close to the peel had the lower fungal severity (Fig. 3A). Rot incidence was evaluated in inoculated slices with *R. stolonif*er since measuring severity for this type of pathogen is not an easy task mainly due to the black and loose mycelium with white aerial fruiting structures (Sardella et al., 2016). Slice 'I' had the highest incidence of *R. stolonifer* (Fig. 3B).

### 3.6 Relationship between tissue composition and susceptibility to major postharvest pathogens

In order to know which variables were characteristics of each slice and determine those that were key to differentiate slices, a first multivariate analysis considering all the analyzed variables, except those of fungal susceptibility to *P. expansum* and *R. stolonifer*, was done. A dendogram graph was used to further obtain a global overview of the relationship between ethylene emission, respiration, sugars, organic acids, antioxidants, phenols and the profile of volatile compounds in a reduced dimension plot. In this data set, 42 variables were used (Fig. 4A). The hierarchical heatmap showed that slices 'I' and 'IV' had similar amounts of the components in cluster 1 (C1), except for sucrose and hexanal (Fig. 4B). This cluster encompasses some major pear character-impact compounds such as butyl butanoate. Components encompassed in cluster 3 (C3) had a similar behavior in slices 'II' and 'III', except for 1-hexanol.

On the other hand, and given the different susceptibility of the different slices locations to blue mold and soft rot, two partial least square regression (PLS) models were performed in order to identify which variables had higher correlation with the susceptibility of *P. expansum* and *R. stolonifer* growth. The PLS models were done to correlate respectively *P. expansum* growth ($Y_1$ variable) and *R. stolonifer* growth ($Y_2$ variable) with a set of potentially explanatory variables: sugars and organic acids content, ethylene production, respiration, dry matter, volatiles compounds, antioxidant capacity and total phenolic content (X variables). Based on PLS method, the X data set was reduced to two principal factors. The first factor explained more than 99% for both fungi, *P. expansum* (Fig. 5A) and *R. stolonifer* (Fig. 6A). The correlation between measured and predicted blue mold severity and soft rot incidence were higher than 0.99, demonstrating the goodness of the model (Fig. 5B, 6B). *P. expansum* growth showed a positively correlation with the sucrose content and some VOC's such as (E)-2-hexenyl acetate, ethyl octanoate, pentyl hexanoate, hexanal, 1-butanol, 2-methyl-1-butanol and 6-methyl-5-hepten-2-one (Fig. 5C). With such a background, 'II' and 'III' slices followed by 'IV' and 'A' were more prone to the growth of this fungus. However, *R. stolonifer* was positively correlated with fructose, malic acid and dry matter content and with ethyl acetate, butyl hexanoate, 2-ethyl-hexanal, butyl hexanoate, (Z)-b-farnesene and α-farnesene (Fig. 6C). 'I' is the most suitable slice for its fungus to growth.

### 3.7 Antifungal efficacy *in vitro* of VOCs against *P. expansum* and *R. stolonifer*

Based on our PLS results (Fig. 5 and 6), 2-ethyl-hexanal and butyl hexanoate were the most effective compounds against *P. expansum* and hexanal and 1-butanol against *R. stolonifer* and their effects were further studied *in vitro* with different concentrations (Fig. s2). The *in vitro* results of exogenous applied compounds, commonly emitted by 'Conference' pears, and their capacity to suppress the mycelial growth of both pathogens is shown in Table 1. All tested oncentrations of 2-ethyl-hexanal, completely controlled *P. expansum* growth while control fruit had a diameter growth of 3 cm after 3 d (Fig. s2A). Moreover, any used concentration of butyl hexanoate was capable to completely control mycelial growth (Fig. s2B). A concentration of 0.22 µL $mL^{-1}$ of hexanal completely controlled the infection (Fig. s2C) and hexanal had an $EC_{50}$ of 0.055 µL $mL^{-1}$ on *R. stolonifer* growth (Table 1). Soft rot was completely controlled by 1-butanol application at 1.76 µL $mL^{-1}$ (Fig. s2D). These results agreed with those found by Neri et al. (2006), who investigated the effect of nine plant volatiles *in vitro* and *in vivo* trials against blue mold on pears and found that *trans*-2-hexanal and carvacol had prominent effects, while hexanal had a less marked effect. Sáenz-Garza et al. (2013) also reported that the hexanal released from microcapsules on the surface of PDA inhibit blue mold growth and it was viable to preserve apple slices. As reviewed by Mari et al. (2016), other aldehydes and alcohols such as benzaldehyde and ethanol have shown promising results controlling different fungal growth in a wide range of fruit and vegetables and hence future studies are warrant.

## 4 Conclusions

The results from this study demonstrate that flavor components including sugars and organic acids are non-uniformly distributed along the flesh of Conference pears. Not only components but also the capacity of the tissue to produce ethylene and $CO_2$ was different along the equatorial location. Some VOCs also presented significant differences among slices. *In vitro* experiments showed that components naturally present along the pear flesh had antifungal activity. Thus, 2-ethyl-hexanal revealed an antifungal effect against *P. expansum* while hexanal and 1-butanol acted against *R. stolonifer*. Overall, the results presented herein give added value to the fresh-cut industry (fruit with improved nutritional quality and flavor) and could improve food security using natural compounds capable of inhibiting major postharvest pathogens.

## Acknowledgements

The authors acknowledge the financial support of the Secretary of Universities and Research of Business and Knowledge Department of Generalitat de Catalunya. Thanks are also given to the CERCA program from the Generalitat de Catalunya. The authors would also like to thank Neus Lamarca and Albert Estévez for his technical assistance.

## References


Alexander, B., Browse, D.J., Reading, S.J., Benjamin, I.S., 1999. A simple and accurate mathematical method for calculation of the EC50. J. Pharmacol. Toxicol. Methods 41, 55–58. https://doi.org/10.1016/S1056-8719(98)00038-0

Alla, M.A.A., El-Sayed, H.Z., Riad, S.E.M., 2008. Control of rhizopus rot disease of apricot fruits (Prunus armeniaca L.) by some plant volatiles aldehydes. Res. J. Agric. Biol. Sci. 4, 424–433.

Aubert, C., Milhet, C., 2007. Distribution of the volatile compounds in the different parts of a white-fleshed peach (Prunus persica L. Batsch). Food Chem. 102, 375–384. https://doi.org/10.1016/j.foodchem.2006.05.030

Benzie, I.F.F., Szeto, Y.T., 1999. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. J. Agric. Food Chem. 47, 633–636. https://doi.org/10.1021/jf9807768

Brahem, M., Renard, C.M.G.C., Eder, S., Loonis, M., Ouni, R., Mars, M., Le Bourvellec, C., 2017. Characterization and quantification of fruit phenolic compounds of European and Tunisian pear cultivars. Food Res. Int. 95, 125–133. https://doi.org/10.1016/J.FOODRES.2017.03.002

Chun, O.K., Kim, D.O., Smith, N., Schroeder, D., Han, J.T., Chang, Y.L., 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. J. Sci. Food Agric. 85, 1715–1724. https://doi.org/10.1002/jsfa.2176

Clark S., Jung S., L.B., 2014. Food processing: principles and applications. John Wiley & Sons.

Colaric, M., Franci-Stampar, A., Hudina, M., 2006. Influence of branch bending on sugar, organic acid and phenolic content in fruits of 'Williams' pears (Pyrus communis L.). J. Sci. Food Agric. 86, 2463–2467. https://doi.org/10.1002/jsfa

Colaric, M., Stampar, F., Hudina, M., 2007. Content levels of various fruit metabolites in the "Conference" pear response to branch bending. Sci. Hortic. (Amsterdam). 113, 261–266. https://doi.org/10.1016/j.scienta.2007.03.016

Collazo, C., Giné-Bordonaba, J., Aguiló-Aguayo, I., Povedano, I., Bademunt, A., Viñas, I., 2018. *Pseudomonas graminis* strain CPA-7 differentially modulates the oxidative response in fresh-cut 'Golden delicious' apple depending on the storage conditions. Postharvest Biol. Technol. 138, 46–55. https://doi.org/10.1016/j.postharvbio.2017.12.013

Costa, G., Noferini, M., Andreotti, C., 2015. Non-destructive determination of internal quality in intact pears by near infrared spectroscopy. Acta Hortic. 821–825. https://doi.org/10.17660/actahortic.2002.596.142

Defilippi, B.G., Manríquez, D., Luengwilai, K., González-Agüero, M., 2009. Chapter 1. Aroma volatiles: biosynthesis and mechanisms of modulation during fruit ripening. Adv. Bot. Res. 50, 1–37. https://doi.org/10.1016/S0065-2296(08)00801-X

El Hadi, M.A.M., Zhang, F.J., Wu, F.F., Zhou, C.H., Tao, J., 2013. Advances in fruit aroma volatile research. Molecules 18, 8200–8229. https://doi.org/10.3390/molecules18078200

Famiani, F., Casulli, V., Baldicchi, A., Battistelli, A., Moscatello, S., Walker, R.P., 2012. Development and metabolism of the fruit and seed of the Japanese plum Ozark premier (Rosaceae). J. Plant Physiol. 169, 551–560. https://doi.org/10.1016/J.JPLPH.2011.11.020

Franck, C., Baetens, M., Lammertyn, J., Verboven, P., Davey, M.W., Nicolaï, B.M., 2003. Ascorbic acid concentration in cv. Conference pears during fruit development and postharvest storage. J. Agric. Food Chem. 51, 4757–4763. https://doi.org/10.1021/jf026229a

Galvis Sánchez, A.C., Gil-Izquierdo, A., Gil, M.I., 2003. Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. J. Sci. Food Agric. 83, 995–1003. https://doi.org/10.1002/jsfa.1436

Giné-Bordonaba, J., Terry, L.A., 2016. Effect of deficit irrigation and methyl jasmonate application on the composition of strawberry (Fragaria x ananassa) fruit and leaves. Sci. Hortic. (Amsterdam). 199, 63–70. https://doi.org/10.1016/j.scienta.2015.12.026

Giné Bordonaba, J., Cantin, C.M., Larrigaudière, C., López, L., López, R., Echeverria, G., 2014. Suitability of nectarine cultivars for minimal processing: the role of genotype, harvest season and maturity at harvest on quality and sensory attributes. Postharvest Biol. Technol. 93. https://doi.org/10.1016/j.postharvbio.2014.02.007

Giné Bordonaba, J., Terry, L.A., 2010. Manipulating the taste-related composition of strawberry fruits (Fragaria × ananassa) from different cultivars using deficit irrigation. Food Chem. 122, 1020–1026. https://doi.org/10.1016/J.FOODCHEM.2010.03.060

Giné Bordonaba, J., Terry, L.A., 2008. Biochemical profiling and chemometric analysis of seventeen UK-grown black currant cultivars. J. Agric. Food Chem. 56, 7422–7430. https://doi.org/10.1021/jf8009377

Goliáš, J., Kožíšková, J., Létal, J., 2015. Changes in volatiles during cold storage and subsequent shelf-life of "Conference" pears treated with 1-MCP. Acta Hortic. 1079, 465–471. https://doi.org/10.17660/ActaHortic.2015.1079.61

Gotor-Vila, A., Teixidó, N., Di Francesco, A., Usall, J., Ugolini, L., Torres, R., Mari, M., 2017. Antifungal effect of volatile organic compounds produced by Bacillus amyloliquefaciens CPA-8 against fruit pathogen decays of cherry. Food Microbiol. 64, 219–225. https://doi.org/10.1016/J.FM.2017.01.006

Hendges, M.V., Neuwald, D.A., Steffens, C.A., Vidrih, R., Zlatić, E., do Amarante, C.V.T., 2018. 1-MCP and storage conditions on the ripening and production of aromatic compounds in Conference and Alexander Lucas pears harvested at different maturity stages. Postharvest Biol. Technol. 146, 18–25. https://doi.org/10.1016/J.POSTHARVBIO.2018.08.006

Ho, Q.T., Hertog, M.L.A.T.M., Verboven, P., Ambaw, A., Rogge, S., Verlinden, B.E., Nicolaï, B.M., 2018. Down-regulation of respiration in pear fruit depends on temperature. J. Exp. Bot. 69, 2049–2060. https://doi.org/10.1093/jxb/ery031

Horvat, R.J., Senter, S.D., Chapman, G.W., Payne, J.A., 1992. Volatiles of ripe Asian pears (Pyrus serotina Rehder). J. Essent. Oil Res. 4, 645–646. https://doi.org/10.1080/10412905.1992.9698151

Hudina, M., Stampar, F., 2000. Sugars and organic acids contents of European (Pyrus communis L.) and Asian (Pyrus serotina Rehd.) pear cultivars. Acta Aliment. 29, 217–230.

Hudina, M., Štampar, F., 2004. Effect of climatic and soil conditions on sugars and organic acids content of pear fruits (Pyrus communis L.) cvs. "Williams" and "Conference." Acta Hortic. 636, 527–531. https://doi.org/10.17660/ActaHortic.2004.636.66

Iglesias, M.B., López, M.L., Echeverría, G., Viñas, I., Zudaire, L., Abadias, M., 2018. Evaluation of biocontrol capacity of Pseudomonas graminis CPA-7 against foodborne pathogens on fresh-cut pear and its effect on fruit volatile compounds. Food Microbiol. 76, 226–236. https://doi.org/10.1016/J.FM.2018.04.007

Imeh, U., Khokhar, S., 2002. Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. J. Agric. Food Chem. 50, 6301–6306. https://doi.org/10.1021/jf020342j

Johnson, J. T., Lennox, J. A., Ujong, U. P., Odey, M. O., Fila, W. O., Edem, P. N., Dasofunjo, K., 2013. Comparative vitamins content of pulp, seed and rind of fresh and dried watermelon (Citrullus Lanatus). Int. J. Sci. Technol. 2.

Knee, M., 1987. Development of ethylene biosynthesis in pear fruits at -1 °C. J. Exp. Bot. 38, 1724–1733. https://doi.org/10.1093/jxb/38.10.1724

Kolniak-Ostek, J., 2016. Chemical composition and antioxidant capacity of different anatomical parts of pear (Pyrus communis L.). Food Chem. 203, 491–497. https://doi.org/10.1016/J.FOODCHEM.2016.02.103

Kou, X., Wang, S., Zhang, Y., Guo, R., Wu, M., Chen, Q., Xue, Z., 2014. Effects of chitosan and calcium chloride treatments on malic acid-metabolizing enzymes and the related gene expression in post-harvest pear cv. 'Huang guan.' Sci. Hortic. (Amsterdam). 165, 252–259. https://doi.org/10.1016/J.SCIENTA.2013.10.034

Lammertyn, J., Franck, C., Verlinden, B.E., Nicolaï, B.M., 2001. Comparative study of the O2, CO2 and temperature effect on respiration between "Conference" pear cell protoplasts in suspension and intact pears. J. Exp. Bot. 52, 1769–1777. https://doi.org/10.1093/jexbot/52.362.1769

Larrigaudière, C., Candan, A.P., Giné-Bordonaba, J., Civello, M., Calvo, G., 2016. Unravelling the physiological basis of superficial scald in pears based on cultivar differences. Sci. Hortic. (Amsterdam). 213, 340–345. https://doi.org/10.1016/j.scienta.2016.10.043

Leontowicz, H., Gorinstein, S., Lojek, A., Leontowicz, M., Íž, M., Soliva-Fortuny, R., Park, Y.S., Jung, S.T., Trakhtenberg, S., Martin-Belloso, O., 2002. Comparative content of some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats. J. Nutr. Biochem. 13, 603–610. https://doi.org/10.1016/S0955-2863(02)00206-1

Li, G., Jia, H., Wu, R., Teng, Y., 2013. Changes in volatile organic compound composition during the ripening of "Nanguoli" pears (Pyrus ussuriensis Maxim) harvested at different growing locations. J. Hortic. Sci. Biotechnol. 88, 563–570. https://doi.org/10.1080/14620316.2013.11513007

Li, Y., Kong, W., Li, M., Liu, H., Zhao, X., Yang, S., Yang, M., 2016. Litsea cubeba essential oil as the potential natural fumigant: inhibition of Aspergillus flavus and AFB1 production in licorice. Ind. Crops Prod. 80, 186–193. https://doi.org/10.1016/J.INDCROP.2015.11.008

Liaudanskas, M., Zymone, K., Viškelis, J., Klevinskas, A., Janulis, V., 2017. Determination of the phenolic composition and antioxidant activity of pear extracts. J. Chem. 2017.

https://doi.org/10.1155/2017/7856521

Lindo-García, V., Larrigaudière, C., Echeverría, G., Murayama, H., Soria, Y., Giné-Bordonaba, J., 2019. New insights on the ripening pattern of 'Blanquilla' pears: a comparison between on- and off-tree ripened fruit. Postharvest Biol. Technol. 150, 112–121. https://doi.org/10.1016/J.POSTHARVBIO.2018.12.013

López, L., Echeverria, G., Usall, J., Teixidó, N., 2015. The detection of fungal diseases in the "Golden Smoothee" apple and "Blanquilla" pear based on the volatile profile. Postharvest Biol. Technol. 99, 120–130. https://doi.org/10.1016/j.postharvbio.2014.08.005

Määttä-Riihinen, K.R., Kamal-Eldin, A., Törrönen, A.R., 2004. Identification and quantification of phenolic compounds in berries of Fragaria and Rubus species (family rosaceae). J. Agric. Food Chem. 52, 6178–6187. https://doi.org/10.1021/jf049450r

Makkumrai, W., Anthon, G.E., Sivertsen, H., Ebeler, S.E., Negre-Zakharov, F., Barrett, D.M., Mitcham, E.J., 2014. Effect of ethylene and temperature conditioning on sensory attributes and chemical composition of "Bartlett" pears. Postharvest Biol. Technol. 97, 44–61. https://doi.org/10.1016/j.postharvbio.2014.06.001

Mari, M., Bautista-Baños, S., Sivakumar, D., 2016. Decay control in the postharvest system: role of microbial and plant volatile organic compounds. Postharvest Biol. Technol. 122, 70–81. https://doi.org/10.1016/j.postharvbio.2016.04.014

Mari, M., Leoni, O., Iori, R., Cembali, T., 2002. Antifungal vapour-phase activity of allyl-isothiocyanate against Penicillium expansum on pears. Plant Pathol. 51, 231–236. https://doi.org/10.1046/j.1365-3059.2002.00667.x

Moriguchi, T., Abe, K., Sanada, T., Yamaki, S., 2019. Levels and role of sucrose synthase, sucrose-phosphate synthase, and acid invertase in sucrose accumulation in fruit of Asian pear. J. Am. Soc. Hortic. Sci. 117, 274–278. https://doi.org/10.21273/jashs.117.2.274

Moya-León, M.A., Vergara, M., Bravo, C., Montes, M.E., Moggia, C., 2006. 1-MCP treatment preserves aroma quality of 'Packham's Triumph' pears during long-term storage. Postharvest Biol. Technol. 42, 185–197. https://doi.org/10.1016/J.POSTHARVBIO.2006.06.003

Neri, F., Donati, I., Veronesi, F., Mazzoni, D., Mari, M., 2010. Evaluation of Penicillium expansum isolates for aggressiveness, growth and patulin accumulation in usual and less common fruit hosts. Int. J. Food Microbiol. 143, 109–117. https://doi.org/10.1016/J.IJFOODMICRO.2010.08.002

Neri, F., Mari, M., Brigati, S., 2006a. Control of Penicillium expansum by plant volatile compounds. Plant Pathol. 55, 100–105. https://doi.org/10.1111/j.1365-3059.2005.01312.x

Neri, F., Mari, M., Menniti, A.M., Brigati, S., Bertolini, P., 2006b. Control of Penicillium expansum in pears and apples by trans-2-hexenal vapours. Postharvest Biol. Technol. 41, 101–108. https://doi.org/10.1016/J.POSTHARVBIO.2006.02.005

Qin, G., Tao, S., Cao, Y., Wu, J., Zhang, H., Huang, W., Zhang, S., 2012. Evaluation of the volatile profile of 33 Pyrus ussuriensis cultivars by HS-SPME with GC–MS. Food Chem. 134, 2367–2382. https://doi.org/10.1016/J.FOODCHEM.2012.04.053

Rapparini, F., Predieri, S., 2003. Pear Fruit Volatiles. John Wiley & Sons.

Rizzolo, A., Cambiaghi, P., Grassi, M., Zerbini, P.E., 2005. Influence of 1-methylcyclopropene and storage atmosphere on changes in volatile compounds and fruit quality of conference pears. J. Agric. Food Chem. 53, 9781–9789. https://doi.org/10.1021/jf051339d

Rudell, D.R., Mattinson, D.S., Fellman, J.K., 2000. The progression of ethylene production and respiration in the tissues of ripening `Fuji'apple fruit. HortScience 35, 1300–1303.

Sáenz-Garza, N.E., Delaquis, P., Durance, T., 2013. Microencapsulation of hexanal by radiant energy vacuum microwave-molecular inclusion for controlled release and inhibition of Penicillium expansum in a model system and on apple tissue. Food Res. Int. 52, 496–502. https://doi.org/10.1016/J.FOODRES.2013.01.040

Saquet, A.A., 2018. Storability of 'Conference' pear under various controlled atmospheres. Erwerbs-Obstbau 60, 275–280. https://doi.org/10.1007/s10341-018-0369-7

Saquet, A.A., 2017. Aroma volatiles of 'Conference' pear and their changes during regular air and controlled atmosphere storage 55–66. https://doi.org/10.26669/2448-4091121

Saquet, A.A., Streif, J., 2017. Respiration rate and ethylene metabolism of 'Jonagold' apple and 'Conference' pear under regular air and controlled atmosphere. Bragantia 76, 335–344. https://doi.org/10.1590/1678-4499.189

Sardella, D., Muscat, A., Brincat, J.P., Gatt, R., Decelis, S., Valdramidis, V., 2016. A comprehensive review of the pear fungal diseases. Int. J. Fruit Sci. 16, 351–377. https://doi.org/10.1080/15538362.2016.1178621

Sha, S., Li, J., Wu, J., Zhang, S., 2011. Characteristics of organic acids in the fruit of different pear species. Afr. J. Agric. Res 6, 2403–2410. https://doi.org/10.5897/AJAR11.316

Sivakumar, D., Bautista-Baños, S., 2014. A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. Crop Prot. 64, 27–37. https://doi.org/10.1016/j.cropro.2014.05.012

Suni, M., Nyman, M., Eriksson, N.A., Björk, L., Björck, I., 2000. Carbohydrate composition and content of organic acids in fresh and stored apples. J. Sci. Food Agric. 80, 1538–1544. https://doi.org/10.1002/1097-0010(200008)80:10<1538::AID-JSFA678>3.0.CO;2-A

Torregrosa, L., Echeverria, G., Illa, J., Giné-bordonaba, J., 2019. Ripening behaviour and consumer acceptance of ‘Conference’ pears during shelf life after long term DCA-storage. Postharvest Biol. Technol. 155, 94–101. https://doi.org/10.1016/j.postharvbio.2019.05.014

Travers, S., Bertelsen, M.G., Petersen, K.K., Kucheryavskiy, S. V., 2014. Predicting pear (cv. Clara Frijs) dry matter and soluble solids content with near infrared spectroscopy. LWT - Food Sci. Technol. 59, 1107–1113. https://doi.org/10.1016/j.lwt.2014.04.048

Veltman, R.., Kho, R.., van Schaik, A.C.., Sanders, M.., Oosterhaven, J., 2000. Ascorbic acid and tissue browning in pears (Pyrus communis L. cvs Rocha and Conference) under controlled atmosphere conditions. Postharvest Biol. Technol. 19, 129–137. https://doi.org/10.1016/S0925-5214(00)00095-8

Veltman, R.H., Sanders, M.G., Persijn, S.T., Peppelenbos, H.W., Oosterhaven, J., 1999. Decreased ascorbic acid levels and brown core development in pears (Pyrus. Physiol. Plant. 39–45.

Villalobos-Acuña, M., Mitcham, E.J., 2008. Ripening of European pears: the chilling dilemma. Postharvest Biol. Technol. 49, 187–200. https://doi.org/10.1016/j.postharvbio.2008.03.003

Wang, H., Cao, G., Prior, R.L., 1996. Total antioxidant capacity of fruits. J. Agric. Food Chem. 44, 701–705. https://doi.org/10.1021/jf950579y

Ward, J.H.J., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58, 236–244.

Zerbini, P.E., Balzarotti, R., Rizzolo, A., Spada, G.L., 1993. Effect of picking date on quality and sensory characteristics of pears after storage and ripening. Acta Hortic. 326.

Zlatić, E., Zadnik, V., Fellman, J., Demšar, L., Hribar, J., Čejić, Ž., Vidrih, R., 2016. Comparative analysis of aroma compounds in “Bartlett” pear in relation to harvest date, storage conditions, and shelf-life. Postharvest Biol. Technol. 117, 71–80. https://doi.org/10.1016/j.postharvbio.2016.02.004

**Supplementary table 1:** Mean ± standard deviations (n=4) values of VOC's concentration (µg $kg^{-1}$) of equatorial slices of 'Conference' pear from different radial locations. Means within the slices preceded by the same small letters are not significantly different at $p \leq 0.05$ (HSD test). No letter indicates the absence of significant differences.

| | Slice | | | |
|---|---|---|---|---|
| **Volatile compounds** | **I** | **II** | **III** | **IV** |
| ***Esters*** | | | | |
| Ethyl Acetate | 7.1±0.8 | 6.6±0.1 | 6.4±0.1 | 5.0±3.3 |
| Tert-Butyl propionate | 3.4±3.0 | 2.4±3.5 | 3.7±2.5 | 2.4±2.9 |
| Methyl butanoate | 1.6±2.9 | 2.4±3.5 | 2.4±2.9 | 1.2±2.5 |
| Butyl acetate | $^{a}$3.6±3.2 | $^{a}$5.5±0.2 | $^{a}$5.5±0.3 | $^{b}$0.0±0.0 |
| Pentyl acetate | 1.2±2.2 | 3.7±0.0 | 1.0±2.1 | 1.3±2.7 |
| Butyl butanoate | 27.2±8.8 | 33.4±3.8 | 29.1±20.9 | 24.6±17.0 |
| Hexyl acetate | 1.3±2.3 | 3.8±0.0 | 2.7±1.9 | 0.0±0.0 |
| 3-Methylbutyl 3-methyl-butanoate | $^{b}$0.0±0.0 | $^{b}$0.0±0.0 | $^{a}$2.7±1.8 | $^{b}$0.0±0.0 |
| (E)-2-Hexenyl acetate | $^{c}$0.0±0.0 | $^{c}$0.0±0.0 | $^{a}$4.1±0.2 | $^{ab}$2.7±1.8 |
| Butyl hexanoate | 4.2±0.1 | 2.0±2.9 | 2.0±2.4 | 3.4±2.4 |
| Hexyl butanoate | $^{b}$0.0±0.0 | $^{b}$0.0±0.0 | $^{b}$0.0±0.0 | $^{a}$2.3±1.6 |
| Hexyl 2-methylbutanoate | $^{b}$0.0±0.0 | $^{a}$3.5±0.0 | $^{b}$0.0±0.0 | $^{b}$0.0±0.0 |
| Ethyl octanoate | 0.0±0.0 | 0.0±0.0 | 2.9±2.0 | 2.7±1.9 |
| Octyl acetate | 2.0±0.0 | 1.0±1.4 | 2.0±0.1 | 1.5±1.0 |
| Pentyl hexanoate | 0.0±0.0 | 0.0±0.0 | 1.2±2.4 | 1.1±2.3 |
| Hexyl hexanoate | 3.0±2.7 | 4.1±0.4 | 4.4±0.5 | 2.2±2.6 |
| ***Alcohols*** | | | | |
| 1-Butanol | 2.7±2.3 | 4.1±0.0 | 4.0±0.1 | 3.0±2.0 |
| 2-Methyl-1-butanol | 3.0±2.7 | 4.6±0.5 | 3.6±2.4 | 3.2±2.2 |
| 1-Hexanol | 3.3±0.1 | 3.6±0.2 | 2.7±1.8 | 3.4±0.1 |
| 2-Ethyl-1-hexanol | 5.0±1.0 | 5.1±0.3 | 5.0±0.8 | 4.2±0.5 |
| 1-Octanol | 1.1±1.9 | 0.0±0.0 | 1.6±1.9 | 0.8±1.6 |
| Benzyl alcohol | 1.4±2.5 | 0.0±0.0 | 3.2±2.2 | 1.0±2.0 |
| ***Aldehydes*** | | | | |
| Hexanal | 104.4±91.9 | 140.2±15.5 | 128.8±29.7 | 128.7±86.3 |
| 2-Ethyl-hexanal | $^{a}$5.1±1.5 | $^{b}$0.0±0.0 | $^{b}$0.0±0.0 | $^{ab}$2.2±2.7 |
| Benzaldehyde | 2.8±2.5 | 4.2±0.2 | 4.2±0.1 | 2.1±2.4 |
| *Terpenoids* | | | | |
| (Z)-β-farnesene | 6.5±6.8 | 5.4±0.0 | 5.9±4.4 | 6.0±4.3 |
| α-farnesene | 4.7±0.3 | 4.4±0.1 | 3.6±2.4 | 3.3±2.2 |
| ***Acid*** | | | | |
| Acetic acid | 8.9±6.0 | 2.8±4.0 | 16.1±27.2 | 5.4±6.4 |
| ***Ketone*** | | | | |
| 6-Methyl-5-hepten-2-one | 2.5±2.3 | 3.8±0.2 | 3.8±0.2 | 2.7±1.8 |

**Table 1:** Antifungal activity of pure volatile organic compounds at different concentrations on the in vitro mycelial growth inhibition (%) tests against *P. expansum* after 5 d and *R. stolonifer* after 3 d. When possible, $EC_{50}$ values were calculated according to Alexander et al. (1999) (mL $L^{-1}$ headspace).

| **Pathogen** | **Compound** | **Concentration (mL $L^{-1}$ headspace)** | **Mycelial growth inhibition (%)** | **$EC_{50}$ (mL $L^{-1}$)** |
|---|---|---|---|---|
| *P. expansum* | 2-Ethyl hexanal | 0.027 | 100.0 | - |
| | | 0.055 | 100.0 | |
| | | 0.11 | 100.0 | |
| | | 0.22 | 100.0 | |
| | | 0.44 | 100.0 | |
| | | 0.88 | 100.0 | |
| | | 1.76 | 100.0 | |
| | Butyl hexanoate | 0.027 | 9.5 | 0.61 |
| | | 0.055 | 29.7 | |
| | | 0.11 | 10.9 | |
| | | 0.22 | 26.6 | |
| | | 0.44 | 37.6 | |
| | | 0.88 | 56.1 | |
| | | 1.76 | 54.9 | |
| *R. stolonifer* | Hexanal | 0.027 | 4.4 | 0.055 |
| | | 0.055 | 50.0 | |
| | | 0.11 | 95.9 | |
| | | 0.22 | 100.0 | |
| | | 0.44 | 100.0 | |
| | | 0.88 | 100.0 | |
| | | 1.76 | 100.0 | |
| | 1-Butanol | 0.027 | ni | 0.48 |
| | | 0.055 | ni | |
| | | 0.11 | ni | |
| | | 0.22 | 9.1 | |
| | | 0.44 | 43.4 | |
| | | 0.88 | 97.6 | |
| | | 1.76 | 100.0 | |

ni: no mycelial growth inhibition observed
-: insufficient data to calculate $EC_{50}$ values.

**List of figures**

**Figure 1:** A) Spatial distribution among slices of dry matter content, B) ethylene production rate, C) $O_2$ consumption rate (black bars, left axis), $CO_2$ production rate (grey bars, left axis) and RQ (○, right axis). Error bars indicate standard deviation for n=4. For each graph, mean values with the same letter are not significantly different according to analysis of variance (ANOVA) and Tukey's HSD test ($P < 0.05$). Horizontal lines represent weighted averages, and were calculated weighting the value at each location by the difference of spherical volumes corresponding to the radius of both extremes of the sample.

**Figure 2:** Contents, referred to unit of pulp fresh mass, of: A) sugars: fructose, glucose and sucrose, and B) acids: malic, citric (black and grey with diagonal lines bars, left axis) and ascorbic (grey dotted bars, right axis), C) antioxidant capacity measured by the FRAP assay and D) total phenolic compounds in different slices of 'Conference' pears spatially distributed. Error bars indicate standard deviation for n=4. For each graph, mean values with the same letter are not significantly different according to analysis of variance (ANOVA) and Tukey's HSD test ($P < 0.05$). Horizontal lines represent weighted averages.

**Figure 3:** Fungal susceptivility, A) blue mold (*Penicillium expansum*) severity and B) soft rot (*Rhizopus stolonifer*) incidence in the different locations of 'Conference' pear flesh. For each graph, mean values with the same letter are not significantly different according to analysis of variance (ANOVA) and Tukey's HSD test ($P < 0.05$).

**Figure 4:** A) Hierarchical heatmap based on the normalized quantities of the analyzed elements and identified volatiles in each 'Conference' section. The lowest content is in the lightest green and the highest in the darkest red. * indicate significant differences ($P < 0.05$) and ** indicate significant differences ($p < 0.01$) between sections. B) Constellation plot of the different clusters.

**Figure 5:** A) Partial Least Squares (PLS) correlation loading plots of the 2 factors of *P. expansum* severity. B) The measured vs the predicted *P. expansum* severity through the model and its correlation coefficient. C) Variable importance plot (VIP), the number of VIP>1.

**Figure 6:** A) Partial Least Squares (PLS) correlation loading plots of the 2 factors of *R. stolonifer* incidence. B) The measured vs the predicted *R. stolonifer* incidence through the model and its correlation coefficient. C) Variable importance plot (VIP), the number of VIP>1.

**Figure supplementary 1:** Methodology used for the equatorial cylinder extraction and slices division in 'Conference' pear. Fruit skin was adhered to the left side of sliceI.

**Figure supplementary 2:** Effects of different concentrations of VOCs, A) 2-ethyl-hexanal and B) butyl hexanoate on mycelia diameter (cm) of *P. expansum* growth during 5 d and C) hexanal and D) 1-Butanol on mycelia diameter (cm) of *R. stolonifer* growth during 3 d. Error bars indicate standard deviation for n=4. For each graph, mean values with the same letter are not significantly different according to analysis of variance (ANOVA) and Tukey's HSD test ($p < 0.05$).

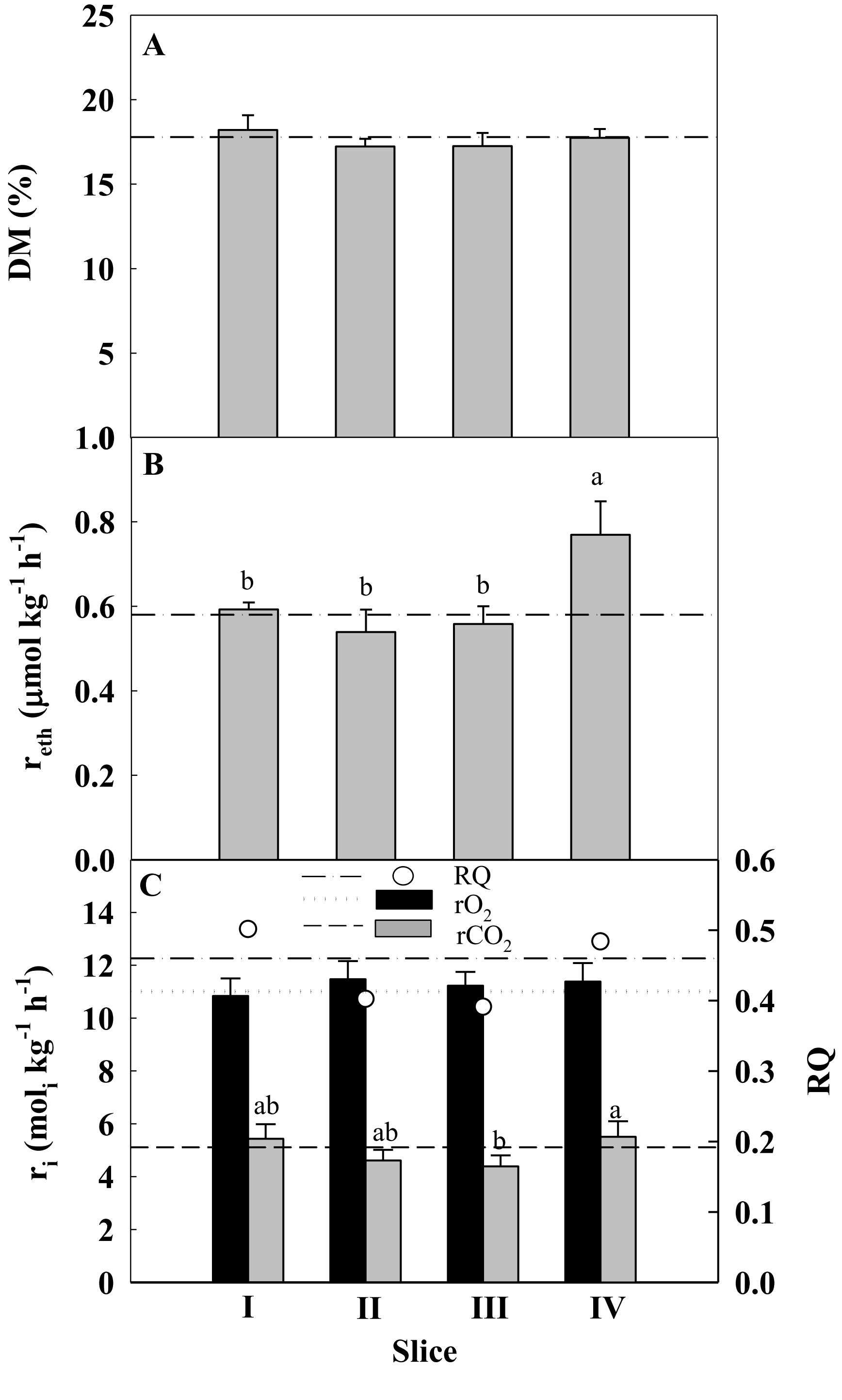

A
B
C
DM (%)
$r_{eth}$ (µmol kg$^{-1}$ h$^{-1}$)
$r_i$ (mol$_i$ kg$^{-1}$ h$^{-1}$)
RQ
RQ
$rO_2$
$rCO_2$
25
20
15
10
5
1.0
0.8
0.6
0.4
0.2
0.0
14
12
10
8
6
4
2
0
0.6
0.5
0.4
0.3
0.2
0.1
0.0
a
b
b
b
ab
ab
b
a
I
II
III
IV
Slice

**Figure 1**

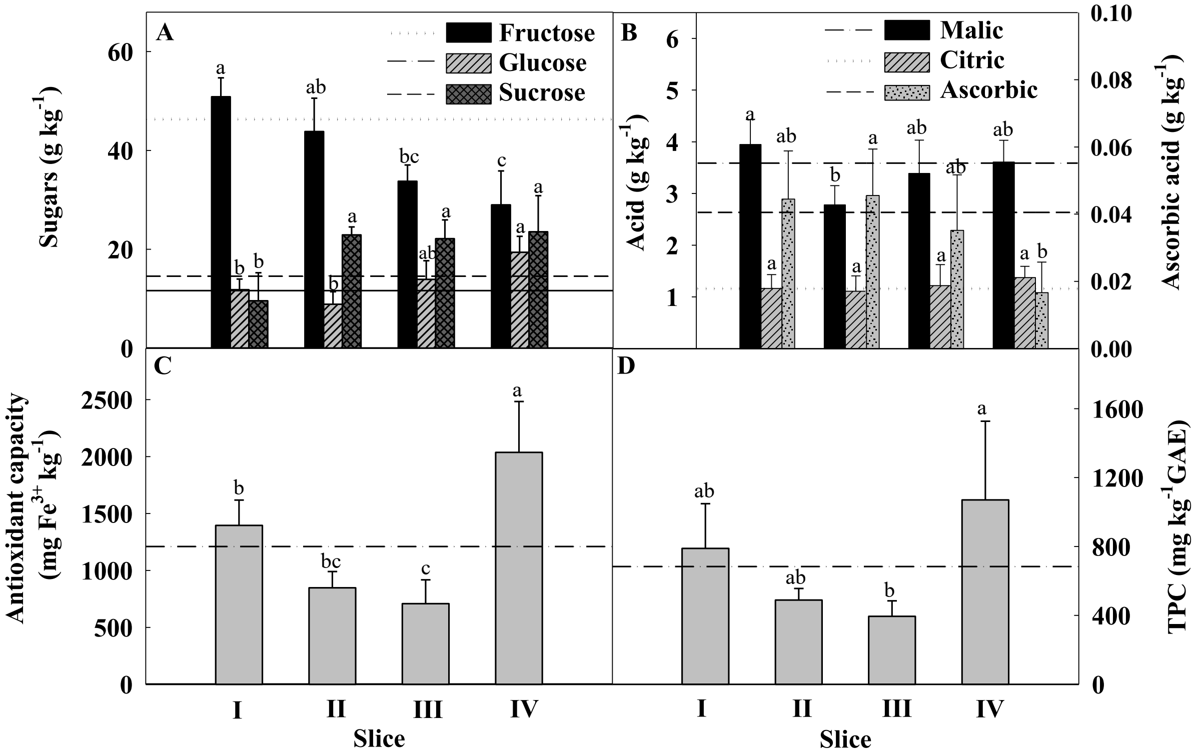

A
B
C
D
Fructose
Glucose
Sucrose
Malic
Citric
Ascorbic
Sugars (g kg-1)
Acid (g kg-1)
Ascorbic acid (g kg-1)
Antioxidant capacity (mg Fe3+ kg-1)
TPC (mg kg-1 GAE)
I
II
III
IV
Slice
Slice

Figure 2

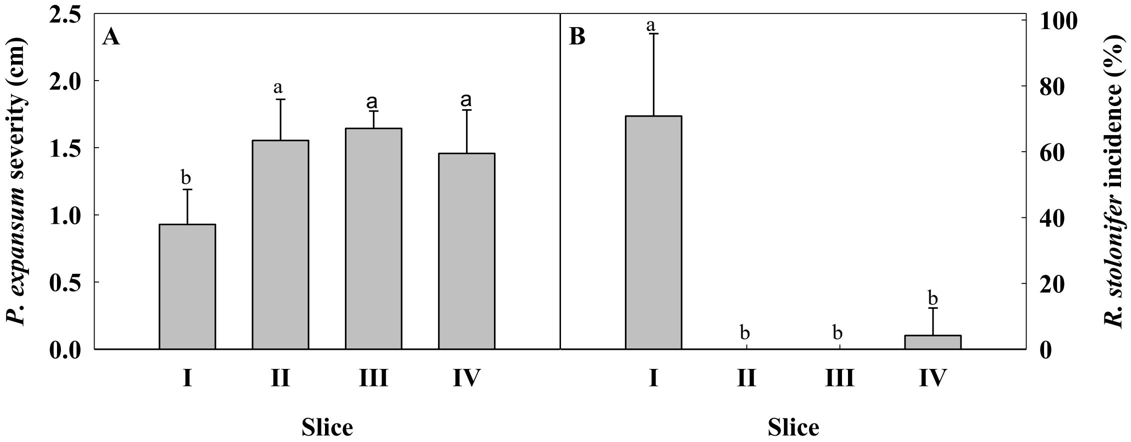
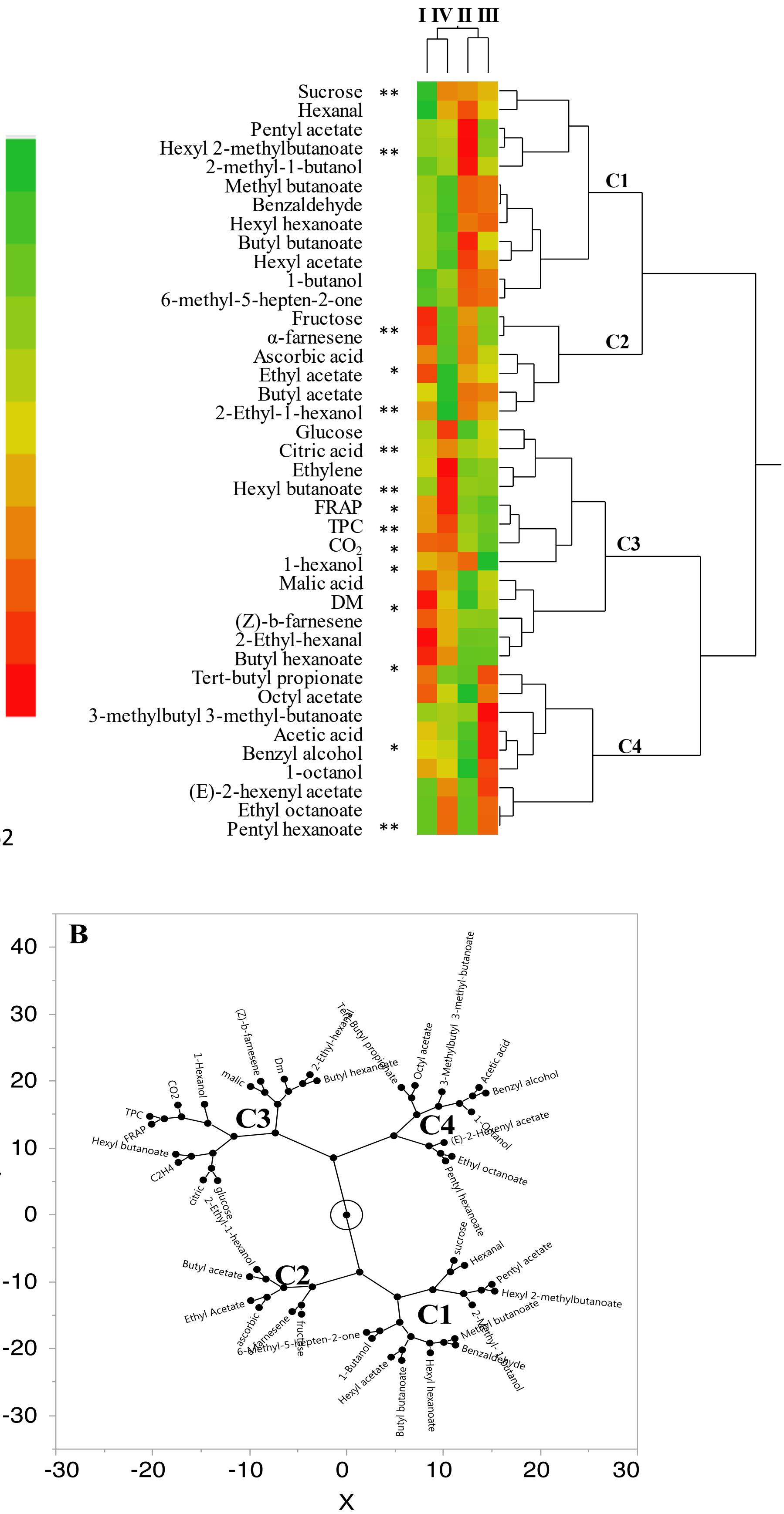
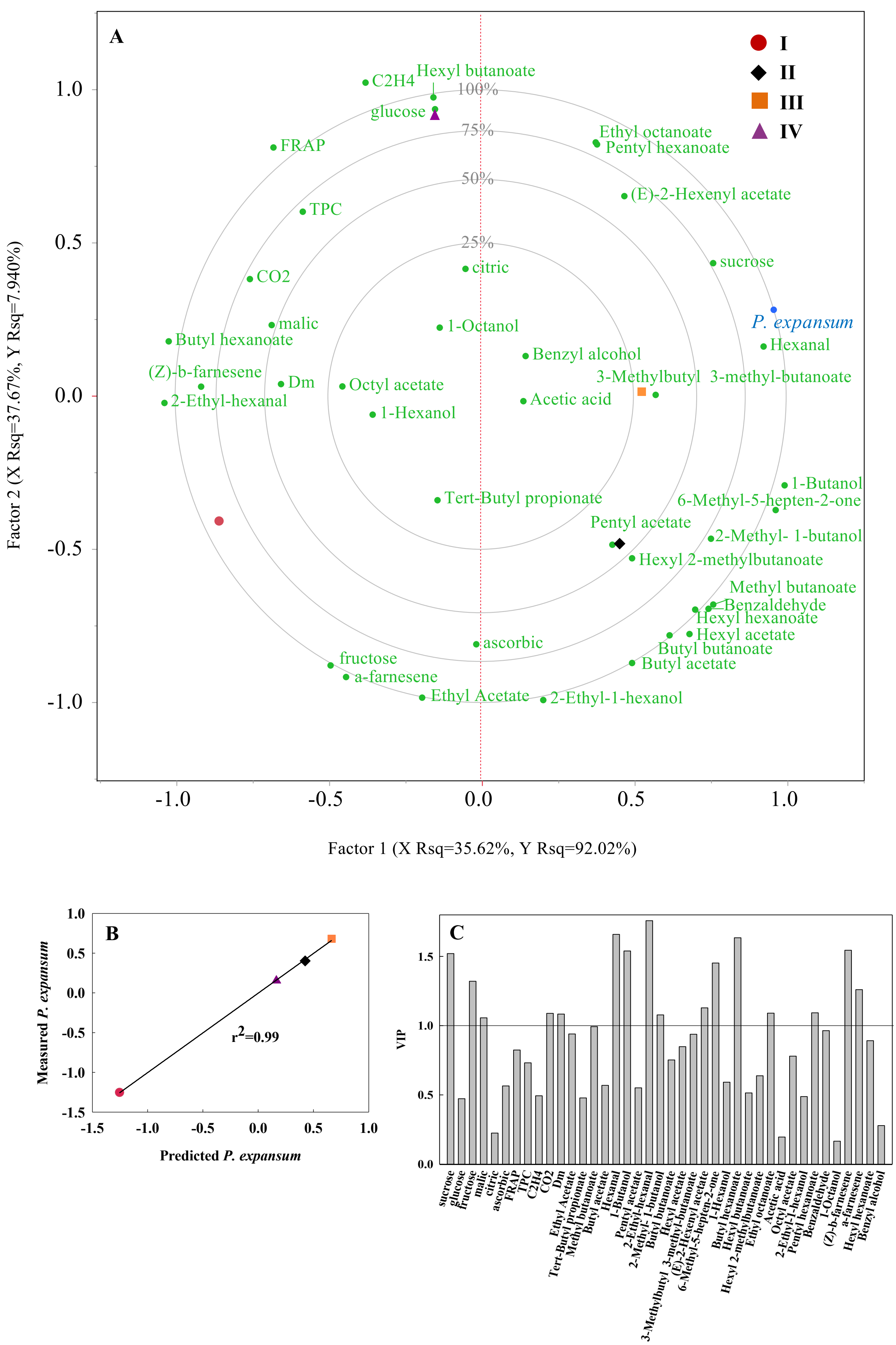

A
B
P. expansum severity (cm)
R. stolonifer incidence (%)
2.5
2.0
1.5
1.0
0.5
0.0
100
80
60
40
20
0
a
b
I
II
III
IV
Slice
Slice

**Figure 3**



I IV II III
Sucrose **
Hexanal
Pentyl acetate
Hexyl 2-methylbutanoate **
2-methyl-1-butanol
Methyl butanoate
Benzaldehyde
Hexyl hexanoate
Butyl butanoate
Hexyl acetate
1-butanol
6-methyl-5-hepten-2-one
Fructose
α-farnesene **
Ascorbic acid
Ethyl acetate *
Butyl acetate
2-Ethyl-1-hexanol **
Glucose
Citric acid **
Ethylene
Hexyl butanoate **
FRAP *
TPC **
$CO_2$ *
1-hexanol *
Malic acid
DM *
(Z)-b-farnesene
2-Ethyl-hexanal
Butyl hexanoate
Tert-butyl propionate *
Octyl acetate
3-methylbutyl 3-methyl-butanoate
Acetic acid
Benzyl alcohol *
1-octanol
(E)-2-hexenyl acetate
Ethyl octanoate
Pentyl hexanoate **
C1
C2
C3
C4
B
40
30
20
10
0
-10
-20
-30
Y
-30 -20 -10 0 10 20 30
X
C1
C2
C3
C4


**Figure 4**



A
I
II
III
IV
Hexyl butanoate
C2H4
glucose
100%
75%
50%
25%
FRAP
TPC
CO2
malic
Butyl hexanoate
(Z)-b-farnesene
Dm
2-Ethyl-hexanal
citric
1-Octanol
Octyl acetate
1-Hexanol
Benzyl alcohol
Acetic acid
3-Methylbutyl 3-methyl-butanoate
Ethyl octanoate
Pentyl hexanoate
(E)-2-Hexenyl acetate
sucrose
P. expansum
Hexanal
1-Butanol
6-Methyl-5-hepten-2-one
Tert-Butyl propionate
Pentyl acetate
2-Methyl- 1-butanol
Hexyl 2-methylbutanoate
Methyl butanoate
Benzaldehyde
Hexyl hexanoate
Hexyl acetate
Butyl butanoate
Butyl acetate
ascorbic
fructose
a-farnesene
Ethyl Acetate
2-Ethyl-1-hexanol
1.0
0.5
0.0
-0.5
-1.0
Factor 2 (X Rsq=37.67%, Y Rsq=7.940%)
Factor 1 (X Rsq=35.62%, Y Rsq=92.02%)
B
r²=0.99
Measured P. expansum
Predicted P. expansum
-1.5
C
VIP
1.5
sucrose
glucose
fructose
malic
citric
ascorbic
FRAP
TPC
C2H4
CO2
Dm
Ethyl Acetate
Tert-Butyl propionate
Methyl butanoate
Butyl acetate
Hexanal
1-Butanol
Pentyl acetate
2-Ethyl-hexanal
2-Methyl-1-butanol
Butyl butanoate
Hexyl acetate
3-Methylbutyl 3-methyl-butanoate
(E)-2-Hexenyl acetate
6-Methyl-5-hepten-2-one
1-Hexanol
Butyl hexanoate
Hexyl butanoate
Hexyl 2-methylbutanoate
Ethyl octanoate
Acetic acid
Octyl acetate
2-Ethyl-1-hexanol
Pentyl hexanoate
Benzaldehyde
1-Octanol
(Z)-b-farnesene
a-farnesene
Hexyl hexanoate
Benzyl alcohol


**Figure 5**

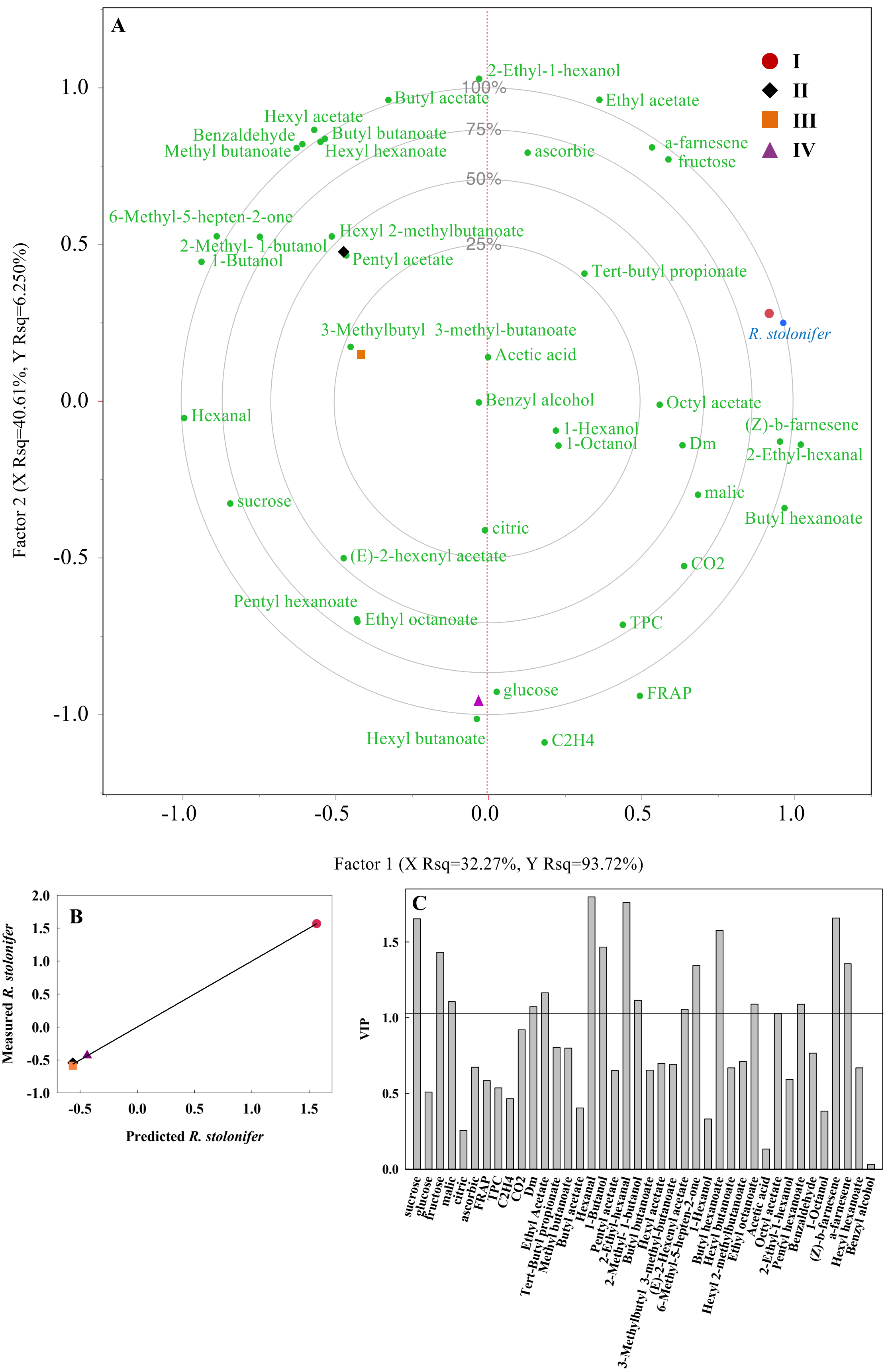

A
I
II
III
IV
1.0
0.5
0.0
-0.5
-1.0
Factor 2 (X Rsq=40.61%, Y Rsq=6.250%)
Factor 1 (X Rsq=32.27%, Y Rsq=93.72%)
100%
75%
50%
25%
2-Ethyl-1-hexanol
Butyl acetate
Ethyl acetate
Hexyl acetate
Benzaldehyde
Butyl butanoate
Methyl butanoate
Hexyl hexanoate
ascorbic
a-farnesene
fructose
6-Methyl-5-hepten-2-one
Hexyl 2-methylbutanoate
2-Methyl- 1-butanol
1-Butanol
Pentyl acetate
Tert-butyl propionate
R. stolonifer
3-Methylbutyl 3-methyl-butanoate
Acetic acid
Benzyl alcohol
Octyl acetate
Hexanal
1-Hexanol
1-Octanol
Dm
(Z)-b-farnesene
2-Ethyl-hexanal
malic
sucrose
Butyl hexanoate
citric
(E)-2-hexenyl acetate
CO2
Pentyl hexanoate
Ethyl octanoate
TPC
glucose
FRAP
Hexyl butanoate
C2H4
B
Measured R. stolonifer
Predicted R. stolonifer
2.0
1.5
-0.5
C
VIP
sucrose
glucose
fructose
malic
citric
ascorbic
FRAP
TPC
C2H4
CO2
Dm
Ethyl Acetate
Tert-Butyl propionate
Methyl butanoate
Butyl acetate
Hexanal
1-Butanol
Pentyl acetate
2-Ethyl-hexanal
2-Methyl-1-butanol
Butyl butanoate
Hexyl acetate
3-Methylbutyl 3-methyl-butanoate
(E)-2-Hexenyl acetate
6-Methyl-5-hepten-2-one
1-Hexanol
Butyl hexanoate
Hexyl butanoate
Hexyl 2-methylbutanoate
Ethyl octanoate
Acetic acid
Octyl acetate
2-Ethyl-1-hexanol
Pentyl hexanoate
Benzaldehyde
1-Octanol
(Z)-b-farnesene
a-farnesene
Hexyl hexanoate
Benzyl alcohol

**Figure 6**

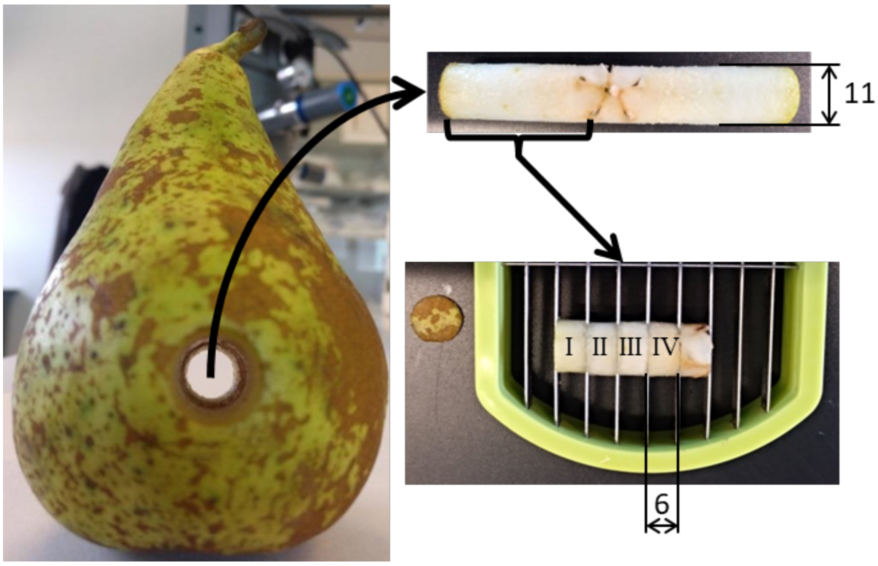
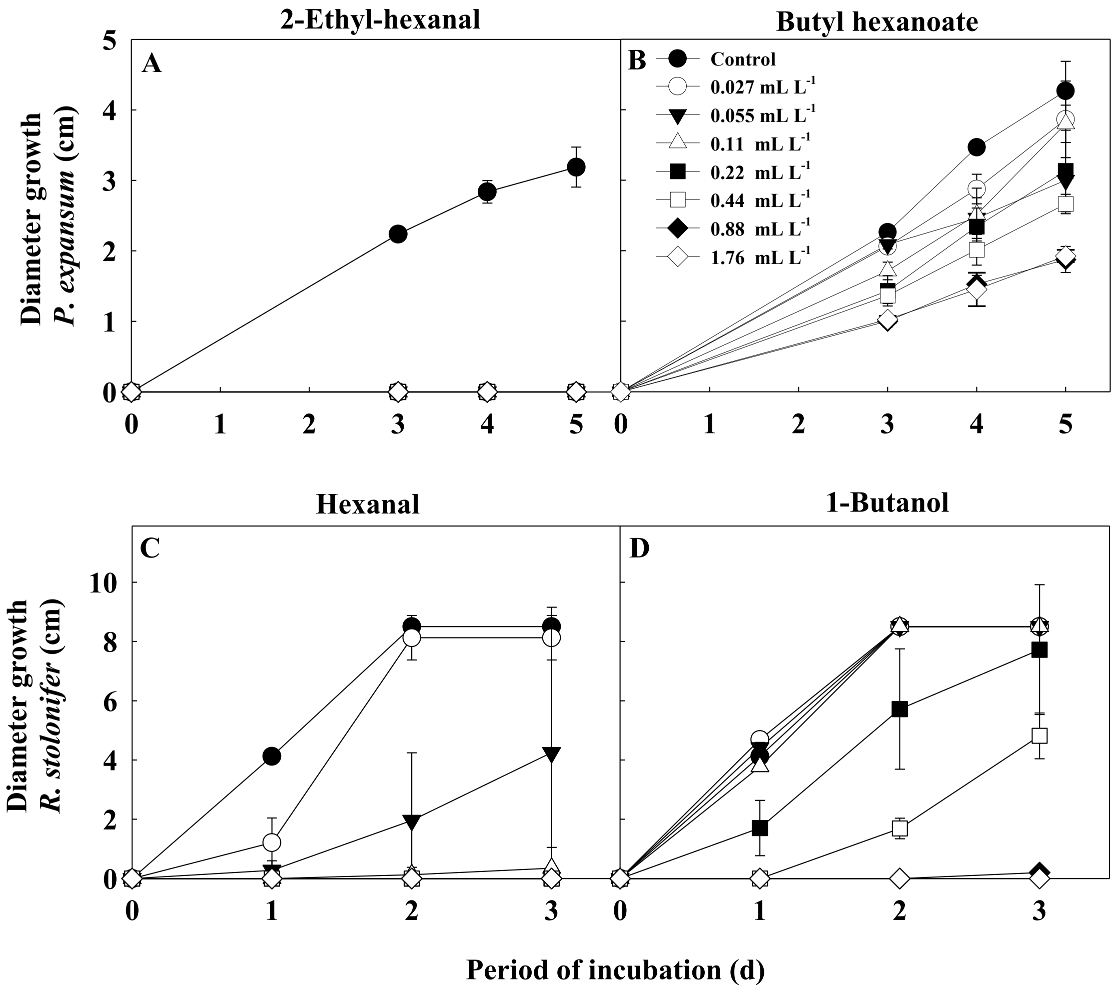



**Figure s1**




783

784 **Figure s2**